HOKKAIDO UNIVERSITY

Title	Antarctic heterotrophic bacterium Hymenobacter nivis P3(T) displays light-enhanced growth and expresses putative photoactive proteins
Author(s)	Terashima, Mia; Ohashi, Keisuke; Takasuka, Taichi E. et al.
Citation	Environmental microbiology reports, 11(2), 227-235 https://doi.org/10.1111/1758-2229.12702
Issue Date	2019-04
Doc URL	https://hdl.handle.net/2115/77213
Type	journal article
File Information	Environmental microbiology reports11(2)_227-235.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

environmental microbiology reports

Environmental Microbiology Reports (2019) **11**(2), 227–235 doi:10.1111/1758-2229.12702

Antarctic heterotrophic bacterium *Hymenobacter nivis* P3^T displays light-enhanced growth and expresses putative photoactive proteins

Mia Terashima,[1]* Keisuke Ohashi,[2] Taichi E. Takasuka,[2] Hisaya Kojima[1] and Manabu Fukui[1]

[1]*Institute of Low Temperature Science, Hokkaido University, Kita-ku, Sapporo, 060-0819, Japan.*

[2]*Research Faculty of Agriculture, Hokkaido University, Kita-ku, Sapporo, 060-8589, Japan.*

Summary

***Hymenobacter nivis* P3^T is a heterotrophic bacterium isolated from Antarctic red snow generated by algal blooms. Despite being non-photosynthetic, *H. nivis* was dominantly found in the red snow environment that is exposed to high light and UV irradiation, suggesting that this species can flourish under such harsh conditions. In order to further understand the adaptive strategies on the snow surface environment of Antarctica, the genome of *H. nivis* P3^T was sequenced and analyzed, which identified genes putatively encoding for light-reactive proteins such as proteorhodopsin, phytochrome, photolyase and several copies of cryptochromes. Culture-based experiments revealed that *H. nivis* P3^T growth was significantly enhanced under light conditions, while dark conditions had increased extracellular polymeric substances. Furthermore, the expression of several putative light-reactive proteins was determined by proteomic analysis. These results indicate that *H. nivis* P3^T is able to potentially utilize light, which may explain its dominance on the red snow surface environment of Antarctica.**

Originality-significance statement

The role of proteorhodopsin in heterotrophic bacteria is not well-characterized, as only a handful of proteorhodopsin-harbouring isolates were shown to have a light-enhanced phenotype through culture-based experiments to date. This is the first study that demonstrates light-stimulated growth and protein expression evidence of photoactive proteins for a non-marine psychrophile and for a member of the genus *Hymenobacter.* It is also the first study that provides comprehensive proteome information for this genus. This study presents significant results in understanding the adaptive mechanism of a heterotrophic non-photosynthetic bacterium thriving on the snow surface environment of Antarctica as well as demonstrating the role of light-utilization in promoting growth, possibly through proteorhodopsin.

Introduction

The snow and ice surface environment of polar and alpine regions can harbour an abundance of microbial life, despite exposure to high light, low temperatures, desiccation and low nutrient levels (Maccario *et al*., 2015; Ordenes-Aenishanslins *et al*., 2016; Anesio *et al*., 2017). Microorganisms flourishing in these environments must tolerate, or even take advantage of such environmental conditions. This can be seen in psychrophilic algal blooms observed seasonally on polar and alpine environments across the world, resulting in vibrant green, orange and red coloration on the snow and ice surface (Hoham and Duval, 2001). These algae utilize light energy for photosynthesis, but are also well-equipped to protect themselves from high light and UV levels (Gorton and Vogelmann, 2003). Interestingly, many heterotrophic microorganisms have been identified in snow and ice surface environments, suggesting that these types of non-photosynthetic organisms can also thrive under these conditions (Weiss, 1983; Lutz *et al*., 2015; Hamilton and Havig, 2017; Terashima *et al*., 2017). These organisms must have strategies to tolerate extreme light conditions of alpine and polar environments, unlike many non-photosynthetic microorganisms that are known to be photosensitive, especially to UV and blue light. Molecules such as porphyrin can absorb light energy and produce reactive oxygen species (ROS), which can in turn cause significant cellular oxidative damage and even lead to cell death (Hamblin and Hasan, 2004; Dai *et al*., 2012; McKenzie *et al*., 2016).

Heterotrophic bacteria belonging to the genus *Hymenobacter*, which is a member of the *Bacteroidetes* phylum, have been isolated from polar environments with high irradiance such as ice, snow, rocks and soil, from the Arctic and Antarctica (Klassen and Foght, 2011; Koo *et al*., 2014; Ahn

Received 10 May, 2018; accepted 2 October, 2018. *For correspondence. E-mail: m.terashima@lowtem.hokudai.ac.jp; Tel. +81-(0) 11-706-6878.

© 2018 Society for Applied Microbiology and John Wiley & Sons Ltd

et al., 2016; Kojima *et al*., 2016; Oh *et al*., 2016; Ordenes-Aenishanslins *et al*., 2016; Sedlacek *et al*., 2017; Jiang *et al*., 2018). Interestingly, *Hymenobacter* species have been isolated from algae-rich environments such as lichen and red-coloured snow, suggesting that these bacteria are able to withstand environments with high light exposure although they are not photosynthetic (Fujii *et al*., 2010; Ahn *et al*., 2016; Kojima *et al*., 2016; Oh *et al*., 2016; Sedlacek *et al*., 2017). Genome sequencing of several species belonging to *Hymenobacter* genus have revealed the presence of several nucleotide excision repair pathways, which could contribute to survival in environments with high irradiation (Collins *et al*., 2000; Zhang *et al*., 2007; Dai *et al*., 2009; Oh *et al*., 2016). In addition, members of the genus *Hymenobacter* accumulate carotenoid pigments, which are known to have antioxidative properties and are thought to play an important adaptive role in environments with high light exposure (Klassen and Foght, 2008).

Hymenobacter nivis P3^T (=DSM 101755^T; =NBRC 111535^T) was isolated from algae-rich red snow in Antarctica during the summer season (Kojima *et al*., 2016). *H. nivis* was found to be by far the most dominating bacterium co-existing with algae on the snow surface, suggesting that this strain is well-adapted to flourish in the coloured snow environment of Antarctica (Fujii *et al*., 2010; Kojima *et al*., 2016). In order to further understand the adaptive strategy behind the success of *H. nivis* in low temperature and light-exposed environments, this study takes a closer look at *H. nivis* P3^T through genome and proteome analyses as well as growth tests. Our results reveal that the growth of *H. nivis* P3^T was enhanced by light and that several putative photoactive proteins were present in its proteome.

Results and discussion

Hymenobacter nivis *P3^T genome contains potential light-reactive proteins*

The genome of *H. nivis* P3^T was sequenced to identify key genes of this snow-dwelling bacterium. Genomic DNA was extracted from 12 ml of *H. nivis* P3^T cells at stationary phase grown at 5°C using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). *H. nivis* cells were grown in Reasoner's 2A (R2A) liquid medium under static, aerobic conditions for all experiments presented in this study. The whole genome of *H. nivis* P3^T was sequenced using PacBio (Takara Bio, Kusatsu, Japan) and the samples were processed and the reads assembled as described previously (Umezawa *et al*., 2016). The sequencing resulted in a total of 63,332 subreads (770,370,754 bp) that were assembled to a single contig of about 5 Mbp in length (GenBank accession number: CP029145) and annotated with the rapid annotation using subsystem technology (RAST) (Supporting Information Table S1) (Aziz *et al*., 2008). The characteristics of the *H. nivis* P3^T genome can be seen in Table 1.

Table 1. Genomic features of *Hymenobacter nivis* P3^T.

Features	
Genome size (bp)	5,027,597
Contig	1
DNA GC content (%)	62.5
Total protein-coding genes	4604
Protein-coding genes with function prediction	2563
Protein-coding genes of hypothetical function	2041
rRNA	9
tRNA	43
Number of subsystems	364
Protein-coding genes in subsystems	1324

After inspection of the genome, *H. nivis* P3^T appears to have genes encoding several light-sensing or light-utilizing genes. The most apparent one is proteorhodopsin (seq4286), a photoactive transmembrane protein found in an array of bacteria and archaea, which acts as a light-mediated ion pump (Sabehi *et al*., 2005; Fuhrman *et al*., 2008; Pinhassi *et al*., 2016). The physiological role of proteorhodopsin is uncharacterized for many bacteria, but it is suggested that a proton-pumping mechanism may play a role in supporting ATP production (Walter *et al*., 2007; Kandori, 2015). The snow surface environment with psychrophilic algal blooms during the summer season of Antarctica, where *H. nivis* P3^T was isolated, is exposed to extreme high light. Although not all species belonging to the *Hymenobacter* genus appear to have the proteorhodopsin gene, its presence could enable this bacterium to utilize light to supplement energy synthesis. Looking closely at the genome of *H. nivis* P3^T, components of a putative resistance/nodulation/division (RND) multidrug efflux transporter (seq4289) is present directly upstream of the proteorhodopsin gene. In previous studies, RND-type efflux pumps were shown to utilize a proton-motive force (pmf) as an energy source in some species, which could also be the case for *H. nivis* P3^T (Piddock, 2006; Alvarez-Ortega *et al*., 2013). Adjacently downstream of the proteorhodopsin, there is a gene encoding for a Brb-like protein (seq4285), which is a putative β-carotene 15,15′-monooxygenase, involved in the generation of the retinal-based photoreceptor of proteorhodopsin. This enzyme is known to cleave β-carotene during retinal synthesis in a single metabolic step and has previously been found to be linked to the presence of proteorhodopsin, along with carotenoid biosynthesis genes (Sabehi *et al*., 2005; Bamann *et al*., 2014). Among the RAST subsystem associated with proteorhodopsin, *H. nivis* P3^T has genes encoding for other proteins involved in carotenoid biosynthesis such as phytoene synthase (seq4567), phytoene dehydrogenase (seq4566, seq3005 and related protein

seq1536), lycopene β-cyclase (seq3242), spheroidene monooxygenase (seq2022) and β-carotene ketolase (seq4572) (Maresca *et al*., 2008; Paniagua-Michel *et al*., 2012).

Aside from proteorhodopsin, other light-reactive proteins that appear to be encoded in the *H. nivis* P3^T genome are photoreceptor genes: two cryptochromes (seq4109 and seq4547) and one phytochrome (seq1249). Photoreceptors act to relay a particular cellular response after a signal transduction triggered by the excitation of chromophores by light (Kottke *et al*., 2018). Cryptochromes contain a flavin chromophore and are found across all branches of life (Brudler *et al*., 2003). These blue-light photoreceptors have been found to be crucial in growth development in plants, whereas in animals, cryptochromes play a role in circadian clocks (Wang *et al*., 2001; Panda *et al*., 2002). Both cryptochromes encoded by *H. nivis* P3^T belong to the DASH (*Drosophila*, *Arabidopsis*, *Synechocystis* and human) family of cryptochromes, which show sequence similarities to photolyases involved DNA repair (Brudler *et al*., 2003). However, bacterial, plant and animal DASH cryptochromes appear to have very weak DNA repair activity on dsDNA, although it should be noted that a recent study on a fungal DASH cryptochrome showed dsDNA repair activity (Selby and Sancar, 2006; Chaves *et al*., 2011; Tagua *et al*., 2015; Sato *et al*., 2018). Currently the biological role that DASH cryptochromes play in prokaryotes, plants or animals is elusive (Chaves *et al*., 2011). Phytochromes, found in the *H. nivis* P3^T genome (seq1249), are another photoreceptor with a bilin chromophore that sense red and far red light, found widely in prokaryotes, plants and fungi (Yeh *et al*., 1997; Rockwell and Lagarias, 2017). In bacteria, phytochromes are known to play a role in the regulation of gene expression, production of pigments and photoprotective responses (Giraud *et al*., 2002; van der Horst *et al*., 2007; Purcell and Crosson, 2008).

The genome of *H. nivis* P3^T also encodes for a deoxyribodipyrimidine photolyase (seq1506), which is similar to a type of photolyase that repairs cyclobutane pyrimidine dimers widespread in bacteria (Sancar *et al*., 1984; Goosen and Moolenaar, 2008). Another DNA repair enzyme, a spore photoproduct lyase (seq1881), appears to be also encoded in the *H. nivis* P3^T genome, although this gene differs from other photolyases as it is not light-dependent (Fajardo-Cavazos *et al*., 1993; Rebeil and Nicholson, 2001; Pieck *et al*., 2006; Goosen and Moolenaar, 2008).

Light enhances growth rates of Hymenobacter nivis *P3*^{T}

After identifying genes that appear to sense or react to the presence of light in the *H. nivis* P3^T genome, such as proteorhodopsin, chryptochrome, phytochrome and photolyase, the growth rates of *H. nivis* P3^T under dark and light conditions were compared, both at 5°C and 15°C (Fig. 1). Pre-cultures for each biological replicate were started from a single colony picked from R2A agar and grown in static liquid R2A medium in the dark until mid-exponential phase (OD_{600} 0.5–0.7). Pre-cultures were diluted to OD_{600} 0.1 with R2A medium for the start of the growth curve analysis. For growth under light conditions, liquid cultures were grown under white fluorescent lights at an intensity of 200 μmol m^2 s^{-1}. Cells were sampled from day 0, every 2 days and fixed in 2% glutaraldehyde and stored at 4°C until use. Cells were stained with the nucleic acid stain SYTO BC dye and counted using Aria BD FACS Aria II flow cytometer with polystyrene microspheres (6 μm diameter) added as an internal standard (Bacteria Counting Kit, Invitrogen, Carlsbad, CA, USA). In addition to forward and side scatters, the cells and beads were monitored by excitation using a 488 nm laser and fluorescence measurement using a

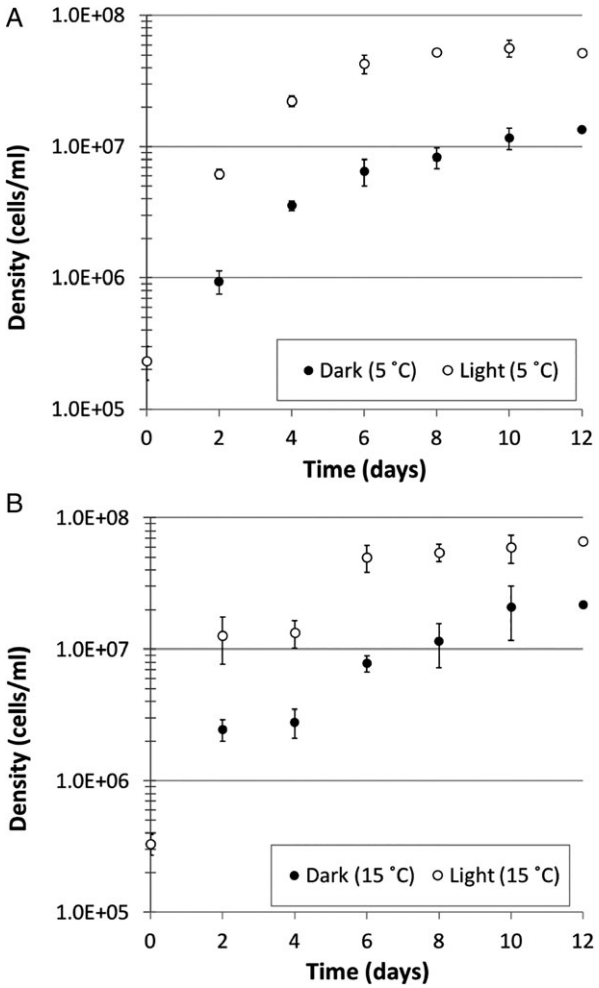

Fig. 1. Growth of *H. nivis* P3^T monitored under dark and light (200 μmol m^2 s^{-1}) conditions at 5°C (panel A) and 15°C (panel B). Error bars indicate the standard deviation of the mean from three biological replicates.

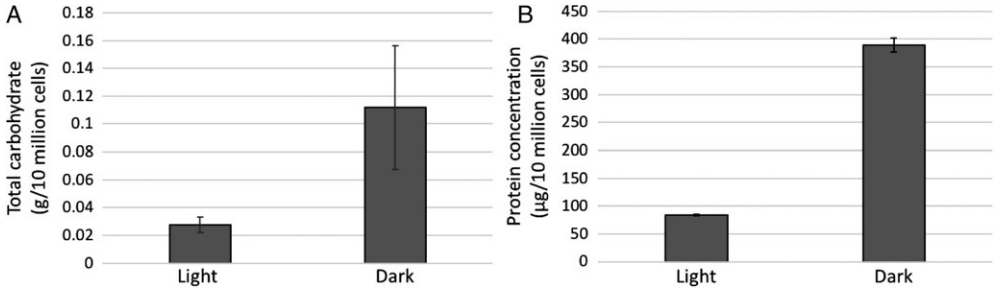

Fig. 2. Total carbohydrate (panel A) and protein (panel B) concentrations from extracellular polymeric substances (EPS) extracted from *H. nivis* P3^T cells at stationary phase after growth at 5°C. P = 1.4×10^{-4} (panel A) and P = 1.8×10^{-6} (panel B) by non-paired two sample *T*-test. Error bars indicate the standard deviation of the mean of three biological replicates.

502 nm long-pass splitter with a 530/30 nm band-pass filter. *H. nivis* P3^T grew without aggregation throughout the conditions used in this study, as confirmed by observation of cells under the microscope and light scatter analysis using flow cytometry.

Under both temperatures, 5°C and 15°C, *H. nivis* P3^T grew at a faster rate and to a higher density in the presence of light compared to dark conditions. The exact mechanism behind this growth enhancement under light remains unknown. However, the presence of proteorhodopsin may contribute to the growth improvement in the light, as one of the possible mechanism of this protein, as mentioned above, is to act as a proton pump, which in turn can promote ATP production (Walter *et al*., 2007). Interestingly, the final cell density in the light condition was higher than that of the dark condition, suggesting that the presence of light increased not only the growth rate, but also its density and capacity. Even though not all proteorhodopsin-harbouring bacteria tested have displayed a light-enhanced growth phenotype (Pinhassi *et al*., 2016), there are members of Bacteroidetes that have increased growth under light such as the marine Flavobacterium *Dokdonia* sp. isolated from the Mediterranean sea and the Antarctic sea-ice psychrophile *Psychroflexus torquis* (Gomez-Consarnau *et al*., 2007; Gomez-Consarnau *et al*., 2010; Feng *et al*., 2013; Gomez-Consarnau *et al*., 2016; Burr *et al*., 2017).

Next, we investigated whether there are substantial differences in the excretion of substances outside of the cell between light and dark growth conditions. Total carbohydrates and protein levels in the extracellular polymeric substances (EPS) were determined with cells that have reached the stationary growth phase. EPS extraction was performed as previously described (Guibaud *et al*., 2005). Briefly, 8 ml of cells in stationary phase, grown under conditions described above, were treated to ultrasonication (40 W, for 2 min) followed by centrifugation (4000 × g, 10 min). The supernatant was collected and passed through a 0.22 μm filter. Total carbohydrate content was determined colorimetrically through the phenol-sulfuric acid method using a kit (#K645, BioVision, Milpitas, CA, USA) and total protein content was determined using the bicinchoninic acid (BCA) method (Pierce BCA protein assay kit, Thermo Fisher Scientific, Waltham, MA, USA). Both carbohydrate levels and protein concentrations were higher under the dark conditions on a per cell basis (Fig. 2), suggesting that light results in more growth in *H. nivis* P3^T, while dark conditions induces a higher accumulation of EPS.

As light enhanced the growth of *H. nivis* P3^T, the question arises of whether light-grown cells are more metabolically active than the dark-grown cells. This was investigated by staining the cells grown under each condition with the RedoxSensor green dye (Thermo Fisher Scientific, Waltham, MA, USA). In this assay, bacterial reductase activity was used as a readout of metabolic activity in the cells, which were co-stained with propidium iodide to control for the presence of dead cells (Kalyuzhnaya *et al*., 2008). Under analysis using flow cytometry, there was a slight increase in green fluorescence of light-grown cells. However, the difference was very subtle, with both dark and light-grown cells displaying overall a similar fluorescence level (Supporting Information Fig. S1). We think that this method was not sensitive enough to detect potential differences in the reductase activities between the two conditions that could have yielded a change in growth rates under light and dark conditions.

Light-reactive proteins are expressed in the proteomes of both dark and light-grown cells

In order to determine whether light-reactive proteins identified in the genome are expressed in *H. nivis* P3^T under our growth conditions, we analysed and compared the proteome of *H. nivis* P3^T grown under dark and light conditions at 5°C. For protein extraction, three biological replicates of 1.5 ml of cells grown in light or dark conditions for 4 days in liquid R2A medium were harvested by centrifugation followed by protein extraction using Minute™ Total Protein Extraction Kit for Microbes with Thick Cell Walls (Invent Biotechnologies, Plymouth, MN, USA).

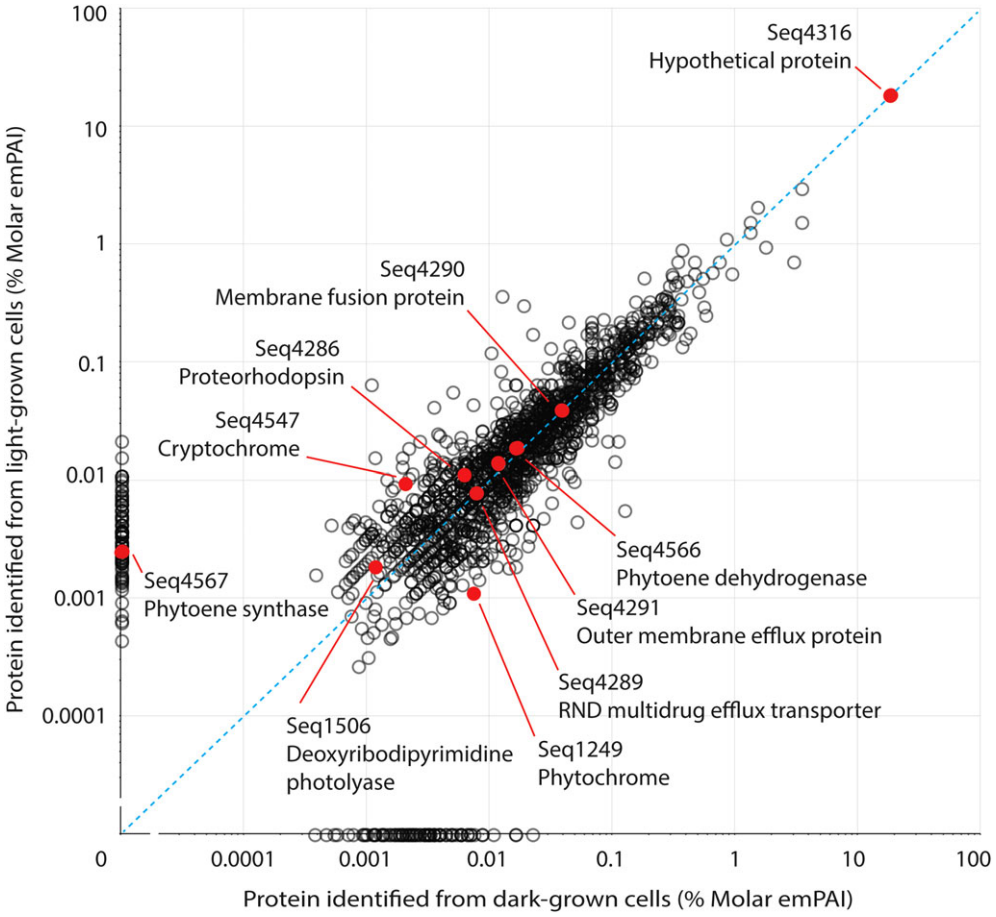

Fig. 3. Proteome of *H. nivis* P3^T under dark and light growth conditions. A total of 1724 proteins are plotted on the graph and the values plotted represent the percentage of molar emPAI for each protein identified with two or more unique peptides. Specific proteins highlighted in the text are marked in red.

The extracted proteins were precipitated by acetone and resuspended in a buffer containing 4.5 M Urea, 25 mM Tris, 0.5% sodium dodecyl sulfate (pH 7). Protein concentrations were determined using the BCA Protein Assay Kit. 20 μg of protein sample was reduced by 25 mM Dithiothreitol for 30 min at 55°C, then was alkylated by 25 mM iodoacetamide for 30 min in the dark. The sample was then digested by a proteomic grade Trypsin (Roche Diagnostics, Risch-Rotkreuz, Switzerland) for overnight at 37°C as described previously (Takasuka *et al*., 2013). The peptides were purified by using a ZipTip-C18 column (Merck, Kenilworth, NJ, USA), and were analysed by a Q-Exactive plus Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) connected to an Easy nLC 1000 liquid chromatograph equipped with C18 column capillary tip column (NTCC-360/75-3-125, Nikkyo Technos, Tokyo, Japan) with a linear gradient raised over 120 min from 5% to 30% acetonitrile in 0.1% formic acid and operated by Xcalibur software (version 3.1, Thermo Fisher Scientific). The Orbitrap full scan mass spectra were obtained at a scan range of 300.0 to 2000.0 m/z and a resolution of 70,000.

MS/MS spectra were used to identify the peptides using Proteome Discoverer 2.0 (Thermo Fisher Scientific) using the coding sequence database for *H. nivis* P3^T described above with the following parameters: 10 ppm peptide mass tolerance, 0.8 Da fragment mass tolerance, and the peptide charge was set at +2, +3 and + 4. The automatic decoy function and percolator function built into the Proteome Discoverer software was employed to obtain accurate and sensitive peptide identifications. Results were filtered to include only proteins identified with 2 or more unique peptide identification with a false discovery rate of less than 1%. The *H. nivis* P3^T proteome for dark and light growth conditions were derived by merging spectral files for the three biological replicates for each condition for peptide identification.

The combined identified proteins from all of the three biological replicates from samples grown under 5°C can be seen in Fig. 3. A total of 1724 proteins were identified (two or more unique peptides in all three biological

replicates from dark or light conditions and less than 1% false discovery rate), with 1598 proteins identified in the dark proteome and 1607 proteins identified in the light proteome (Supporting Information Table S2). Within the 1724 total proteins identified, 79 and 80 proteins were exclusively identified in the dark samples and light samples respectively. Abundance of each protein can be estimated using the exponentially modified protein abundance index (emPAI) (Ishihama *et al*., 2005). This index enables the estimation of protein abundance by normalizing the number of identified peptides belonging to a given protein by the theoretical number of peptides, which allows for comparison of abundance of proteins of different sizes. The emPAI values can be denoted as a molar percent to represent the abundance of a particular protein within the observed proteome (Shinoda *et al*., 2010).

Overall, the proteomes between dark and light conditions were very similar, suggesting that there were no dramatic shifts in protein expression between *H. nivis* $P3^T$ cultured under either condition (Fig. 3). Regarding light-sensitive proteins identified in the genome, proteorhodopsin (seq4286), cryptochrome (seq4547), phytochrome (seq1249), deoxyribodipyrimidine photolyase (seq1506) were detected in both dark and light conditions with similar estimation of abundance (Fig. 3). To note, another putative cryptochrome (seq4109) found in the genome as well as the spore photoproduct lyase (seq1881) were not detected in this proteome set. Among the genes involved in carotenoid biosynthesis, phytoene synthase (seq4567) was detected only in the light proteome, and phytoene dehydrogenase (seq4566) was detected in both conditions. Other proteins involved in carotenoid biosynthesis identified in the genome, such as the lycopene β-cyclase (seq3242), spheroidene monooxygenase (seq2022) and β-carotene ketolase (seq4572) were not identified in the proteome dataset. Interestingly, the Brb-like protein (seq4285), a putative β-carotene 15,15′-monooxygenase downstream to the proteorhodopsin gene was not detected, while the three components making up the RND multidrug efflux transporter upstream from the proteorhodopsin gene were all detected (Venter *et al*., 2015). These include the RND multidrug efflux transporter (seq4289), which is an inner membrane protein, the membrane fusion protein of the RND multidrug efflux pump (seq4290) and the outer membrane efflux protein precursor (seq4291).

Other than the proteins expected to be light-sensitive, some of the most abundant proteins in the *H. nivis* $P3^T$ proteome under both growth conditions were, as expected, proteins involved in protein synthesis, such as ribosomal proteins and elongation factors (Supporting Information Table S2). There are numerous proteins of unknown functions that were highly abundant. One notable protein is a hypothetical protein seq4316, with a percent molar emPAI value at around 18% (Fig. 3). This protein has a predicted size of 24 kDa and appears to be highly conserved among other species belonging to the *Hymenobacter* genus and also in other members of the *Bacteroidetes* phylum and more distantly in a limited number of members in the subphylum *Betaproteobacteria* in the *Burkholderiales* order. Interestingly, the closest member of *Betaproteobacteria* with a protein similar to this hypothetical protein is a psychrophilic strain isolated from an alpine glacier, *Polaromonas glacialis* (Margesin *et al*., 2012; Wang *et al*., 2014).

Among the ~80 proteins exclusively identified in the dark or light proteome, the 20 most abundant proteins for each condition had a percent molar emPAI value ranging from ~0.005 to ~0.02% (Fig. 3, Supporting Information Table S2). For the dark proteome, these included an adenosylcobinamide-phosphate guanylyltransferase (Seq301), a protein involved in adenosylcobalamin (vitamin B_{12}) synthesis, a hypothetical protein with a LysM domain (Seq2793), likely involved in peptidoglycan and chitin binding, and a chitinase (Seq121) involved in chitin breakdown (Lawrence and Roth, 1995; Bhattacharya *et al*., 2007; Buist *et al*., 2008). Proteins identified exclusively in the light proteome included a putative iron or molybdenum transporter (Seq2209), a cytochrome oxidase biogenesis protein with a putative copper metallochaperone function (Seq2199), a guanine deaminase (Seq4171), an enzyme in the nucleotide metabolism, and a peptide methionine sulfoxide reductase (Seq3668), involved in protein repair after oxidative damage (Weissbach *et al*., 2002; Liaw *et al*., 2004; Banci *et al*., 2011). Although these proteins suggest a different physiological state between cells grown in the dark and light, many of the proteins found exclusively in each of the proteome were low in abundance in relation to other proteins present in the *H. nivis* $P3^T$ proteome, suggesting that no major conclusions can be drawn from these low-abundant proteins (Fig. 3). In sum, the proteome dataset demonstrates that many of the genes identified in the genome that are expressed under our growth conditions, which include light-reactive proteins such as proteorhodopsin and proteins of the carotenoid biosynthesis, could explain the light-enhanced growth phenotype and light-tolerance of *H. nivis* $P3^T$.

Conclusions

In this study, we sequenced the genome of a heterotrophic bacterium *Hymenobacter nivis* $P3^T$, originally isolated from Antarctica, which revealed several genes encoding for putative light-reactive proteins. Growth tests at 5°C and 15°C under dark and light conditions showed that growth rates were enhanced under the light, while EPS accumulation was higher in the dark. Further analysis of the proteome of dark- and light-grown cells at 5°C identified that the proteome remains similar between the two conditions. Furthermore, the expression of putative

light-reactive proteins identified in the genome were confirmed as these proteins were present in the proteomes of both conditions, suggesting constitutive expression of these proteins under growth conditions implemented in this study. These identified proteins were: proteorhodopsin, cryptochrome, phytochrome and deoxyribodipyrimidine photolyase. The light-enhanced growth of *H. nivis* $P3^T$ and the expression of proteorhodopsin and other putative light-reactive proteins suggest that this bacterium is likely to sense and utilize light, resulting in a growth advantage under the light-exposed conditions of Antarctic red snow.

Acknowledgements

The flow cytometric analysis was carried with the instrument at the institute for Genetic Medicine provided by Hokkaido University's Open Facility program. This study was supported in part by the Institute of Fermentation, Osaka, and by the grant for Joint Research Program of the Institute of Low Temperature Science. The authors declare no conflict of interest.

References

Ahn, D.H., Han, S.R., Oh, T.J., and Park, H. (2016) Complete genome sequence of ionizing radiation-resistant *Hymenobacter* sp. strain PAMC26628 isolated from an Arctic lichen. *J Biotechnol* **223**: 50–51.

Alvarez-Ortega, C., Olivares, J., and Martinez, J.L. (2013) RND multidrug efflux pumps: what are they good for? *Front Microbiol* **4**: 7.

Anesio, A.M., Lutz, S., Chrismas, N.A.M., and Benning, L.G. (2017) The microbiome of glaciers and ice sheets. *NPJ Biofilms Microbiomes* **3**: 10.

Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A., *et al*. (2008) The RAST server: rapid annotations using subsystems technology. *BMC Genomics* **9**: 75.

Bamann, C., Bamberg, E., Wachtveitl, J., and Glaubitz, C. (2014) Proteorhodopsin. *Biochim Biophys Acta* **1837**: 614–625.

Banci, L., Bertini, I., Cavallaro, G., and Ciofi-Baffoni, S. (2011) Seeking the determinants of the elusive functions of Sco proteins. *FEBS J* **278**: 2244–2262.

Bhattacharya, D., Nagpure, A., and Gupta, R.K. (2007) Bacterial chitinases: properties and potential. *Crit Rev Biotechnol* **27**: 21–28.

Brudler, R., Hitomi, K., Daiyasu, H., Toh, H., Kucho, K., Ishiura, M., *et al*. (2003) Identification of a new cryptochrome class. Structure, function, and evolution. *Mol Cell* **11**: 59–67.

Buist, G., Steen, A., Kok, J., and Kuipers, O.P. (2008) LysM, a widely distributed protein motif for binding to (peptido) glycans. *Mol Microbiol* **68**: 838–847.

Burr, D.J., Martin, A., Maas, E.W., and Ryan, K.G. (2017) In situ light responses of the proteorhodopsin-bearing Antarctic Sea-ice bacterium, Psychroflexus torques. *ISME J* **11**: 2155–2158.

Chaves, I., Pokorny, R., Byrdin, M., Hoang, N., Ritz, T., Brettel, K., *et al*. (2011) The cryptochromes: blue light photoreceptors in plants and animals. *Annu Rev Plant Biol* **62**: 335–364.

Collins, M.D., Hutson, R.A., Grant, I.R., and Patterson, M.F. (2000) Phylogenetic characterization of a novel radiation-resistant bacterium from irradiated pork: description of *Hymenobacter actinosclerus* sp. nov. *Int J Syst Evol Microbiol* **50**: 731–734.

Dai, J., Wang, Y., Zhang, L., Tang, Y., Luo, X., An, H., and Fang, C. (2009) *Hymenobacter tibetensis* sp. nov., a UV-resistant bacterium isolated from Qinghai-Tibet plateau. *Syst Appl Microbiol* **32**: 543–548.

Dai, T., Gupta, A., Murray, C.K., Vrahas, M.S., Tegos, G.P., and Hamblin, M.R. (2012) Blue light for infectious diseases: Propionibacterium acnes, *Helicobacter pylori*, and beyond? *Drug Resist Updat* **15**: 223–236.

Fajardo-Cavazos, P., Salazar, C., and Nicholson, W.L. (1993) Molecular cloning and characterization of the *Bacillus subtilis* spore photoproduct lyase (spl) gene, which is involved in repair of UV radiation-induced DNA damage during spore germination. *J Bacteriol* **175**: 1735–1744.

Feng, S., Powell, S.M., Wilson, R., and Bowman, J.P. (2013) Light-stimulated growth of proteorhodopsin-bearing sea-ice psychrophile Psychroflexus torquis is salinity dependent. *ISME J* **7**: 2206–2213.

Fuhrman, J.A., Schwalbach, M.S., and Stingl, U. (2008) Proteorhodopsins: an array of physiological roles? *Nat Rev Microbiol* **6**: 488–494.

Fujii, M., Takano, Y., Kojima, H., Hoshino, T., Tanaka, R., and Fukui, M. (2010) Microbial community structure, pigment composition, and nitrogen source of red snow in Antarctica. *Microb Ecol* **59**: 466–475.

Giraud, E., Fardoux, J., Fourrier, N., Hannibal, L., Genty, B., Bouyer, P., *et al*. (2002) Bacteriophytochrome controls photosystem synthesis in anoxygenic bacteria. *Nature* **417**: 202–205.

Gomez-Consarnau, L., Gonzalez, J.M., Riedel, T., Jaenicke, S., Wagner-Dobler, I., Sanudo-Wilhelmy, S.A., and Fuhrman, J.A. (2016) Proteorhodopsin light-enhanced growth linked to vitamin-B1 acquisition in marine *Flavobacteria*. *ISME J* **10**: 1102–1112.

Gomez-Consarnau, L., Gonzalez, J.M., Coll-Llado, M., Gourdon, P., Pascher, T., Neutze, R., *et al*. (2007) Light stimulates growth of proteorhodopsin-containing marine *Flavobacteria*. *Nature* **445**: 210–213.

Gomez-Consarnau, L., Akram, N., Lindell, K., Pedersen, A., Neutze, R., Milton, D.L., *et al*. (2010) Proteorhodopsin phototrophy promotes survival of marine bacteria during starvation. *PLoS Biol* **8**: e1000358.

Goosen, N., and Moolenaar, G.F. (2008) Repair of UV damage in bacteria. *DNA Repair (Amst)* **7**: 353–379.

Gorton, H.L., and Vogelmann, T.C. (2003) Ultraviolet radiation and the snow alga *Chlamydomonas nivalis* (Bauer) Wille. *Photochem Photobiol* **77**: 608–615.

Guibaud, G., Comte, S., Bordas, F., Dupuy, S., and Baudu, M. (2005) Comparison of the complexation potential of extracellular polymeric substances (EPS), extracted from activated sludges and produced by pure bacteria strains, for cadmium, lead and nickel. *Chemosphere* **59**: 629–638.

Hamblin, M.R., and Hasan, T. (2004) Photodynamic therapy: a new antimicrobial approach to infectious disease? *Photochem Photobiol Sci* **3**: 436–450.

Hamilton, T.L., and Havig, J. (2017) Primary productivity of snow algae communities on stratovolcanoes of the Pacific Northwest. *Geobiology* **15**: 280–295.

Hoham, R.W., and Duval, B. (2001) Microbial ecology of snow and freshwater ice with emphasis on snow algae. In *Snow Ecology: An Interdisciplinary Examination of Snow-Covered Ecosystems*. Jones, H.G., Pomeroy, J.W., Walker, D.A., and Hoham, R.W. (eds). New York: Cambridge University Press, pp. 168–228.

Ishihama, Y., Oda, Y., Tabata, T., Sato, T., Nagasu, T., Rappsilber, J., and Mann, M. (2005) Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein. *Mol Cell Proteomics* **4**: 1265–1272.

Jiang, F., Danzeng, W., Zhang, Y., Zhang, Y., Jiang, L., Liu, J., *et al*. (2018) *Hymenobacter rubripertinctus* sp. nov., isolated from Antarctic tundra soil. *Int J Syst Evol Microbiol* **68**: 663–668.

Kalyuzhnaya, M.G., Lidstrom, M.E., and Chistoserdova, L. (2008) Real-time detection of actively metabolizing microbes by redox sensing as applied to methylotroph populations in Lake Washington. *ISME J* **2**: 696–706.

Kandori, H. (2015) Ion-pumping microbial rhodopsins. *Front Mol Biosci* **2**: 52.

Klassen, J.L., and Foght, J.M. (2008) Differences in carotenoid composition among hymenobacter and related strains support a tree-like model of carotenoid evolution. *Appl Environ Microbiol* **74**: 2016–2022.

Klassen, J.L., and Foght, J.M. (2011) Characterization of *Hymenobacter isolates* from Victoria upper glacier, Antarctica reveals five new species and substantial non-vertical evolution within this genus. *Extremophiles* **15**: 45–57.

Kojima, H., Watanabe, M., Tokizawa, R., Shinohara, A., and Fukui, M. (2016) *Hymenobacter nivis* sp. nov., isolated from red snow in Antarctica. *Int J Syst Evol Microbiol* **66**: 4821–4825.

Koo, H., Ptacek, T., Crowley, M., Swain, A.K., Osborne, J. D., Bej, A.K., and Andersen, D.T. (2014) Draft genome sequence of *Hymenobacter* sp. strain IS2118, isolated from a freshwater Lake in Schirmacher oasis, Antarctica, reveals diverse genes for adaptation to cold ecosystems. *Genome Announc* **2**: e00739–e00714.

Kottke, T., Xie, A., Larsen, D.S., and Hoff, W.D. (2018) Photoreceptors take charge: emerging principles for light sensing. *Annu Rev Biophys* **47**: 291–313.

Lawrence, J.G., and Roth, J.R. (1995) The cobalamin (coenzyme B12) biosynthetic genes of *Escherichia coli*. *J Bacteriol* **177**: 6371–6380.

Liaw, S.H., Chang, Y.J., Lai, C.T., Chang, H.C., and Chang, G.G. (2004) Crystal structure of *Bacillus subtilis* guanine deaminase: the first domain-swapped structure in the cytidine deaminase superfamily. *J Biol Chem* **279**: 35479–35485.

Lutz, S., Anesio, A.M., Field, K., and Benning, L.G. (2015) Integrated 'Omics', targeted metabolite and single-cell analyses of Arctic snow algae functionality and adaptability. *Front Microbiol* **6**: 1323.

Maccario, L., Sanguino, L., Vogel, T.M., and Larose, C. (2015) Snow and ice ecosystems: not so extreme. *Res Microbiol* **166**: 782–795.

Maresca, J.A., Graham, J.E., and Bryant, D.A. (2008) The biochemical basis for structural diversity in the carotenoids of chlorophototrophic bacteria. *Photosynth Res* **97**: 121–140.

Margesin, R., Sproer, C., Zhang, D.C., and Busse, H.J. (2012) Polaromonas glacialis sp. nov. and Polaromonas cryoconiti sp. nov., isolated from alpine glacier cryoconite. *Int J Syst Evol Microbiol* **62**: 2662–2668.

McKenzie, K., Maclean, M., Grant, M.H., Ramakrishnan, P., MacGregor, S.J., and Anderson, J.G. (2016) The effects of 405 nm light on bacterial membrane integrity determined by salt and bile tolerance assays, leakage of UV-absorbing material and SYTOX green labelling. *Microbiology* **162**: 1680–1688.

Oh, T.J., Han, S.R., Ahn, D.H., Park, H., and Kim, A.Y. (2016) Complete genome sequence of Hymenobacter sp. strain PAMC26554, an ionizing radiation-resistant bacterium isolated from an Antarctic lichen. *J Biotechnol* **227**: 19–20.

Ordenes-Aenishanslins, N., Anziani-Ostuni, G., Vargas-Reyes, M., Alarcon, J., Tello, A., and Perez-Donoso, J.M. (2016) Pigments from UV-resistant Antarctic bacteria as photosensitizers in dye sensitized solar cells. *J Photochem Photobiol B* **162**: 707–714.

Panda, S., Hogenesch, J.B., and Kay, S.A. (2002) Circadian rhythms from flies to human. *Nature* **417**: 329–335.

Paniagua-Michel, J., Olmos-Soto, J., and Ruiz, M.A. (2012) Pathways of carotenoid biosynthesis in bacteria and microalgae. *Methods Mol Biol* **892**: 1–12.

Piddock, L.J. (2006) Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clin Microbiol Rev* **19**: 382–402.

Pieck, J.C., Hennecke, U., Pierik, A.J., Friedel, M.G., and Carell, T. (2006) Characterization of a new thermophilic spore photoproduct lyase from Geobacillus stearothermophilus (SplG) with defined lesion containing DNA substrates. *J Biol Chem* **281**: 36317–36326.

Pinhassi, J., DeLong, E.F., Beja, O., Gonzalez, J.M., and Pedros-Alio, C. (2016) Marine bacterial and archaeal ion-pumping Rhodopsins: genetic diversity, physiology, and ecology. *Microbiol Mol Biol Rev* **80**: 929–954.

Purcell, E.B., and Crosson, S. (2008) Photoregulation in prokaryotes. *Curr Opin Microbiol* **11**: 168–178.

Rebeil, R., and Nicholson, W.L. (2001) The subunit structure and catalytic mechanism of the Bacillus subtilis DNA repair enzyme spore photoproduct lyase. *Proc Natl Acad Sci U S A* **98**: 9038–9043.

Rockwell, N.C., and Lagarias, J.C. (2017) Phytochrome diversification in cyanobacteria and eukaryotic algae. *Curr Opin Plant Biol* **37**: 87–93.

Sabehi, G., Loy, A., Jung, K.H., Partha, R., Spudich, J.L., Isaacson, T., *et al*. (2005) New insights into metabolic properties of marine bacteria encoding proteorhodopsins. *PLoS Biol* **3**: e273.

Sancar, A., Smith, F.W., and Sancar, G.B. (1984) Purification of *Escherichia coli* DNA photolyase. *J Biol Chem* **259**: 6028–6032.

Sato, R., Harada, R., and Shigeta, Y. (2018) The binding structure and affinity of photodamaged duplex DNA with members of the photolyase/cryptochrome family: a computational study. *Biophys Physicobiol* **15**: 18–27.

Sedlacek, I., Kralova, S., Kyrova, K., Maslanova, I., Busse, H.J., Stankova, E., *et al*. (2017) Red-pink pigmented *Hymenobacter coccineus* sp. nov., *Hymenobacter lapidarius* sp. nov. and *Hymenobacter glacialis* sp. nov., isolated from rocks in Antarctica. *Int J Syst Evol Microbiol* **67**: 1975–1983.

Selby, C.P., and Sancar, A. (2006) A cryptochrome/photolyase class of enzymes with single-stranded DNA-specific photolyase activity. *Proc Natl Acad Sci U S A* **103**: 17696–17700.

Shinoda, K., Tomita, M., and Ishihama, Y. (2010) emPAI Calc—for the estimation of protein abundance from large-scale identification data by liquid chromatography-tandem mass spectrometry. *Bioinformatics* **26**: 576–577.

Tagua, V.G., Pausch, M., Eckel, M., Gutierrez, G., Miralles-Duran, A., Sanz, C., *et al*. (2015) Fungal cryptochrome with DNA repair activity reveals an early stage in cryptochrome evolution. *Proc Natl Acad Sci U S A* **112**: 15130–15135.

Takasuka, T.E., Book, A.J., Lewin, G.R., Currie, C.R., and Fox, B.G. (2013) Aerobic deconstruction of cellulosic biomass by an insect-associated Streptomyces. *Sci Rep* **3**: 1030.

Terashima, M., Umezawa, K., Mori, S., Kojima, H., and Fukui, M. (2017) Microbial community analysis of colored snow from an alpine snowfield in northern Japan reveals the prevalence of Betaproteobacteria with snow algae. *Front Microbiol* **8**: 1481.

Umezawa, K., Watanabe, T., Miura, A., Kojima, H., and Fukui, M. (2016) The complete genome sequences of sulfur-oxidizing Gammaproteobacteria Sulfurifustis variabilis skN76(T) and Sulfuricaulis limicola HA5(T). *Stand Genomic Sci* **11**: 71.

van der Horst, M.A., Key, J., and Hellingwerf, K.J. (2007) Photosensing in chemotrophic, non-phototrophic bacteria: let there be light sensing too. *Trends Microbiol* **15**: 554–562.

Venter, H., Mowla, R., Ohene-Agyei, T., and Ma, S. (2015) RND-type drug e ffl ux pumps from Gram-negative bacteria: molecular mechanism and inhibition. *Front Microbiol* **6**: 377.

Walter, J.M., Greenfield, D., Bustamante, C., and Liphardt, J. (2007) Light-powering *Escherichia coli* with proteorhodopsin. *Proc Natl Acad Sci U S A* **104**: 2408–2412.

Wang, H., Ma, L.G., Li, J.M., Zhao, H.Y., and Deng, X.W. (2001) Direct interaction of *Arabidopsis* cryptochromes with COP1 in light control development. *Science* **294**: 154–158.

Wang, Z., Chang, X., Yang, X., Pan, L., and Dai, J. (2014) Draft genome sequence of *Polaromonas glacialis* strain R3-9, a *Psychrotolerant bacterium* isolated from Arctic glacial foreland. *Genome Announc* **2**: e00695–e00614.

Weiss, R.L. (1983) Fine-structure of the snow alga (*Chlamydomonas nivalis*) and associated bacteria. *J Phycol* **19**: 200–204.

Weissbach, H., Etienne, F., Hoshi, T., Heinemann, S.H., Lowther, W.T., Matthews, B., *et al*. (2002) Peptide methionine sulfoxide reductase: structure, mechanism of action, and biological function. *Arch Biochem Biophys* **397**: 172–178.

Yeh, K.C., Wu, S.H., Murphy, J.T., and Lagarias, J.C. (1997) A cyanobacterial phytochrome two-component light sensory system. *Science* **277**: 1505–1508.

Zhang, Q., Liu, C., Tang, Y., Zhou, G., Shen, P., Fang, C., and Yokota, A. (2007) *Hymenobacter xinjiangensis* sp. nov., a radiation-resistant bacterium isolated from the desert of Xinjiang, China. *Int J Syst Evol Microbiol* **57**: 1752–1756.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Details of the *Hymenobacter nivis* P3^T genome annotation by RAST. Abbreviations used are: PEG, protein encoding gene; RNA, RNA encoding gene; and FIGfam, freely available protein families. The abbreviations found in the evidence code column are: ISU, unique feature/function in the genome; ICW, feature clustered in a number of features in the genome; FF, belongs in a FIGfam. Please refer to the previous publication introducing the RAST program for details of the annotation methods (Aziz *et al*., 2008).

Table S2. The proteome of *Hymenobacter nivis* P3^T grown under dark and light conditions. Results from three biological replicates from each condition were merged.

Fig. S1. Dark- and light-grown *H. nivis* P3^T cells stained with Bac*Light* RedoxSensor Green dye sensitive to bacterial reductase activity to monitor metabolic activity, and propidium iodide to identify dead cells, followed by analysis by flow cytometry. 10,000 cells are plotted on the graph in pseudocolor. Red and grey lines have been added to allow for easier comparison of the distribution of the population. The red dashed line represents the highest density region for the distribution of dark-grown cells. Green RedoxSensor fluorescence was monitored using 488 nm excitation laser and a 530/30 nm emission filter and propidium iodide fluorescence was monitored using 488 nm excitation laser and a 695/40 nm emission filter.